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## Osmoregulation in juvenile and adult white sturgeon, *Acipenser transmontanus*

Maryann McEnroe & Joseph J. Cech, Jr.

Departments of Zoology and Wildlife and Fisheries Biology, University of California, Davis, CA 95616,  
U.S.A.

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### Synopsis

Blood samples from cannulated young adult (2.5–15 kg) white sturgeon, acclimated to San Francisco Bay water (24 ppt) had plasma values of  $248.8 \pm 13.5$  mOsm kg<sup>-1</sup> H<sub>2</sub>O,  $[Na^+] = 125 \pm 8.0$  mEq l<sup>-1</sup>,  $[K^+] = 2.6 \pm 0.8$  mEq l<sup>-1</sup> and  $[Cl^-] = 122 \pm 3.0$  mEq l<sup>-1</sup>. Freshwater acclimated sturgeon had an osmolality of  $236 \pm 7$ ,  $[Na^+] = 131.6 \pm 4.4$ ,  $[K^+] = 2.5 \pm 0.7$  and  $[Cl^-] = 110.6 \pm 3.6$ . Freshwater acclimated fish gradually exposed to sea water (increase of 5 ppt h<sup>-1</sup>) had higher plasma osmolalities than did the bay water acclimated fish. These young adult sturgeon are able to tolerate transfer from fresh water to sea water as well as gradual transfer from sea water to fresh water. Plasma electrolytes in transferred fish are regulated, but tend to differ from long term acclimated fish at the same salinities. There is a gradual increase in the upper salinity tolerance (abrupt transfer) of juvenile white sturgeon with weight: 5–10 ppt for 0.4–0.9 g fish, 10–15 ppt for 0.7–1.8 g fish, and 15 ppt for 4.9–50.0 g fish. The ability of juveniles to regulate plasma osmolality is limited. The young adult fish are able to tolerate higher salinities (35 ppt) than juvenile sturgeon but probably are also characterized by low activity of the necessary ion exchange mechanisms in the gills which permit rapid adjustment of blood electrolytes with graduate change in external salinity.

### Introduction

*Acipenser transmontanus* is a chondrosteian, a group of ancient actinopterygian fish which evolved at least 200 million years ago. *A. transmontanus* is found along the Pacific coast of North America from British Columbia to California and is anadromous, migrating up the larger rivers along the coast (the Fraser, Columbia, Umpqua, and Sacramento Rivers) to spawn. It is believed that these fish reach sexual maturity between fifteen and twenty years of age, and may spawn every two to ten years thereafter, depending on age and physiological state (Doroshov 1985). In the fall these sturgeon enter the estuaries associated with the

rivers and slowly migrate upstream over the next several months. Spawning occurs during the winter months (February–March). Sturgeon juveniles remain in fresh water from several months to several years, depending on the species (Doroshov 1985). However, it is not known how long *A. transmontanus* juveniles remain in a freshwater environment, or when they start their seaward migration. Thus, the natural history of *A. transmontanus* suggests several interesting physiological problems: How do they osmoregulate? When can the juveniles successfully tolerate sea water?

Although there have been many investigations of osmoregulation in adult teleosts, there have been fewer on the more 'primitive' fish groups.

This is especially true of the Chondrostei, as a result of their large size, and the difficulty of obtaining them. Three previous studies on sturgeon osmoregulation (Magnin 1962; Urist & Van de Putte 1967, Potts & Rudy 1972) have indicated that sturgeon are hypo/hyperosmotic regulators, as are the teleosts. However, a paucity of data and a lack of documentation regarding environmental conditions prompt further studies into chondrosteian osmoregulation. This is especially true concerning the development of osmoregulatory capabilities in young fish. Virtually all of the studies in this area have been focused on the anadromous salmonids, as a result of their extensive hatchery culture and economic importance.

In order to determine how adult white sturgeon osmoregulate, and how this capacity for osmoregulation changes with development, our study was composed of two parts: a study of plasma electrolytes of adult specimens, and a study of salinity tolerance in juveniles.

## Materials and methods

### *Studies on adult sturgeon*

Twenty adult *Acipenser transmontanus* were snagged in San Francisco Bay at the initiation of the spawning migration during the winters of 1981-1982 and 1982-83. The fish were of both sexes and early stages of gonadal development as determined after experimentation. After capture, the fish were transported to the Tiburon Laboratory of the National Marine Fisheries Service, and placed in tanks with flow-through, aerated, bay water having a salinity of 22-26 parts per thousand (ppt).

During the winter of 1981-1982 all experiments were conducted at the Tiburon Laboratory. Ten sturgeon were held in fiberglass tanks (2.14 m × 0.91 m × 0.50 m depth) with a constant flow of San Francisco Bay water (12-14°C) having a salinity range of 22-26 ppt. The water temperature (YSI Model 51B thermistor), salinity (AO Goldberg refractometer), and dissolved oxygen (YSI Model 51B electrode and meter) were monitored frequently. The fish were allowed to recover from the

stress of capture for one week, and then were cannulated as described below. These fish were used to obtain the bay water acclimated electrolyte values.

The following winter (1982-1983) ten sturgeon (*A. transmontanus*) were caught as previously described and slowly acclimated to fresh water over several days. They were then transported from Tiburon, California, to the Institute of Ecology at the University of California, Davis (UCD) in fresh water. In Davis, the fish were allowed to recover from the stress of transport, and to further acclimate to fresh water, for another two weeks. They were then cannulated as described below and placed in the fiberglass tanks with a flow-through supply of non-chlorinated fresh water (11-14°C) and allowed to recover for 2-3 days before the initiation of the experiment. The temperature, salinity, and dissolved oxygen of the water were monitored daily. Upon initiation of the experiment initial freshwater acclimated blood samples were taken (as described below) and the plasma frozen for later analysis. The salinity was then increased from fresh water (0 ppt) to sea water (33-35 ppt) over a two-day period (increases of 5 ppt h<sup>-1</sup>) by starting a flow of dissolved and aerated Instant Ocean brine into the tank (11-14°C). On the first day of the experiment the salinity was increased to 22 ppt over eight h and left at that salinity for 24 h. Blood samples were taken at each end of this 24 h period at 22 ppt. The next day the flow of the brine solution was again initiated, and the salinity increased to 33-35 ppt over several hours. The fish were kept at 33-35 ppt for three days in a static system. Blood samples were taken when the salinity reached 33-35 ppt and then taken every 24 h for the next 3 d. The water was gradually replaced with fresh 'sea water' on the second day. At the end of the 3 d period the salinity was decreased to 0 ppt (5 ppt h<sup>-1</sup>) and blood samples taken. Blood samples were then taken after 24 h and one week in fresh water.

### *Cannulation technique*

The fish were allowed to recover from the stress of capture and/or transport for at least several days

prior to ventral aortic or ventricular cannulation. The fish to be cannulated was placed ventral surface up into a fabric stretcher having a hood at one end for the sturgeon's head. A hose carrying water for gill ventilation was placed into the fish's mouth. In this position the fish would usually remain still. No anesthetic was used. An eighteen gauge, thin-wall 5 cm hypodermic (cannulation) needle was inserted dorsally at the isthmus, into the ventral aorta or ventricle. A slight negative pressure on the attached syringe pulled deoxygenated blood into the syringe, and a pulsatile flow was observed. At this point the syringe was removed, and approximately 5 cm of the 1.3 m length of PE 50 cannula tubing (0.580 mm I.D. x 0.965 mm O.D.) was inserted through the cannulation needle. The cannulation needle was then removed over the cannula tubing which was filled with a heparinized saline solution (ammonium heparin in 0.9% saline). The blood in the cannula was flushed back into the fish with the heparinized saline. The cannula was secured to the ventral surface and side of the fish with sutures so that the distal portion of the cannula floated in the water. After ensuring that blood could be drawn through the cannula, it was refilled with heparinized saline and tied off, and the fish returned to its tank.

The cannulation technique proved effective for many of the experimental specimens. Many completed the increase/decrease in salinity cycle successfully and were subsequently released into San Francisco Bay or the Sacramento River. However, in several specimens the cannula pulled out during the experiment, limiting the data gathered on these fish.

#### *Blood sampling technique*

Since the purpose of the cannulation was to obtain repeated blood samples without stressing the fish, care was taken not to disturb or excite the fish when blood samples were taken. Blood samples were taken by cutting the knot on the cannula, removing the saline, and attaching a pre-heparinized (dry ammonium heparin crystals) syringe and 23 gauge needle to the cannula. Blood samples of 2 ml volume were taken during the winter of 1981-1982,

and 1 ml samples during the winter of 1982-1983.

After the samples were obtained, micro-hematocrits were determined and the rest of the blood was centrifuged at 4500 rpm for 5 min. Most of the plasma was removed and frozen. The remaining plasma and packed red blood cells were remixed and injected into the fish, after the saline had been removed from the cannula. Thus, repeated blood samples could be obtained without significantly altering either the oxygen carrying capacity or the buffering capacity of the blood.

#### *Electrolyte determinations*

The frozen plasma samples were later thawed and then immediately analyzed for plasma sodium, potassium, chloride, and osmolality. Plasma sodium and potassium were analyzed using a IL 143 flame photometer, chloride with Radiometer CMT-10 chloride titrator, and osmolality with a Wescor 5100B vapor pressure osmometer.

#### *Studies on juvenile sturgeon*

Juvenile white sturgeon were obtained from several spawns of wild caught sturgeon at the aquaculture facility at the Institute of Ecology, UCD. The fish were transported to our laboratory in the Department of Wildlife and Fisheries Biology, UCD. They were maintained in fiberglass tanks having a continuous flow of aerated, stripped, and non-chlorinated well water until use. The fish were fed the diet on which they had been raised (either tubifex worms or Oregon Moist Pellets) supplemented with live brine shrimp.

Upon initiation of the experiment, the fish were weighed in a tared beaker with water. Five weight groups were used: 0.4-0.9 g, 0.7-1.5 g, 0.9-1.8 g, 4.9-9.5 g, and 18.0-56.0 g. The fish were abruptly transferred to an insulated, plastic cooler which contained water of the test salinity at the same temperature as the fresh water tank (17-19°C). Water for the experiments was made up using non-chlorinated well water and Instant Ocean artificial sea salts. The salinity was measured with the refractometer. The water was aerated and mixed overnight before initiation of the experiment.

Ten fish of each size group were tested at each salinity (0 ppt, 5 ppt, 10 ppt, 15 ppt, 25 ppt, 30 ppt, and 35 ppt), and survival was monitored for three days. 'Survival' at the test salinity was determined as survival for three days or longer. This duration was chosen as preliminary experiments indicated that those juvenile sturgeon which survived three days at the test salinity would survive indefinitely at that salinity. In tests where fish survived 24 h, test water was daily replaced with water of the test salinity after feeding. The experiments on the smallest fish (0.4–0.9 g and 0.7–1.5 g) were conducted in March, several weeks after spawning. The 0.9–9.5 g fish and the 18.0–56.0 g fish were run in November, and the following September, respectively.

To test whether pre-acclimation to 10 ppt or 15 ppt for one week would increase survival at higher salinities, 70 fish of the 4.9–9.5 g range were abruptly transferred to the lower salinities (40 fish to 10 ppt and 30 fish to 15 ppt), and maintained for 1 week with daily water changes. At the end of the week-long acclimation period, 10 fish from each group were transferred to water of the same salinity (either 10 ppt or 15 ppt) as well as to higher salinities: 15 ppt, 25 ppt, and 35 ppt. Survival was monitored for three days.

Plasma samples were obtained from fish of the largest size class used (18.0–56.0 g) after 9 h in either 15 ppt or 25 ppt by sacrificing the fish and

obtaining blood by caudal section. The osmolalities of the samples were determined with the vapor pressure osmometer.

## Results

The bay water acclimated sturgeon were hypo-osmotic to bay water, and the freshwater acclimated sturgeon were hyperosmotic to fresh water (Table 1). The plasma osmolality and electrolyte values of the bay water and freshwater acclimated fish were similar (Table 1). No significant difference was found in the plasma concentrations of  $\text{Na}^+$  and  $\text{K}^+$  and the osmolality when comparing bay water acclimated and freshwater acclimated adult sturgeon. There was a significant decrease ( $P < 0.001$ , t-test) in the concentration of  $\text{Cl}^-$  in freshwater acclimated adults compared with bay water acclimated fish (Table 1). When the freshwater acclimated sturgeon were gradually exposed to increased salinity up to sea water over a two day period, and then held in sea water for 3 days there was a continual rise in all the measured plasma variables (Table 2). An analysis of variance showed that there were no significant differences between the freshwater and 22 ppt (24 h) concentrations of  $\text{Na}^+$ ,  $\text{Cl}^-$ , or the osmolality in these fish. However, there were significant differences ( $P < 0.05$ ) between the freshwater and both the initial and 3

Table 1. Plasma electrolyte values ( $\bar{X} \pm \text{SD}$ ) of bay water and freshwater acclimated *Acipenser transmontanus*.

	Salinity (Time at that salinity)		Level of significance
	22–26 ppt (>2 wk) (bay water acclimated)	0 ppt (> several wk) (freshwater acclimated)	
$\text{Na}^+$ (mEq l <sup>-1</sup> )	125.0 $\pm$ 8.0	131.6 $\pm$ 4.4	N.S.
$\text{K}^+$ (mEq l <sup>-1</sup> )	2.6 $\pm$ 0.8	2.5 $\pm$ 0.7	N.S.
$\text{Cl}^-$ (mEq l <sup>-1</sup> )	122.0 $\pm$ 3.0	110.6 $\pm$ 3.6	$P < 0.001$
Osmo (mOsm kg <sup>-1</sup> )	248.8 $\pm$ 13.5	236.1 $\pm$ 7.1	N.S.
Number of fish	7	7	
Osmo (mOsm kg <sup>-1</sup> ) of water	approx. 700	<100	

Sample sizes are smaller than indicated in text due to the unfortunate proclivity of these fish to either jump out of their tank and/or knock out standpipes. In either case, the fish died.

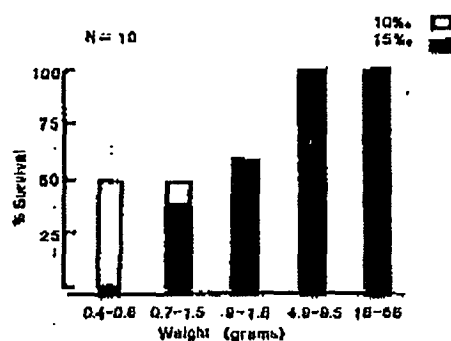


Fig. 1. Percent survival of several size classes of juvenile white sturgeon with abrupt transfer to either 10 ppt (open bars) or 15 ppt (closed bars) salinity.

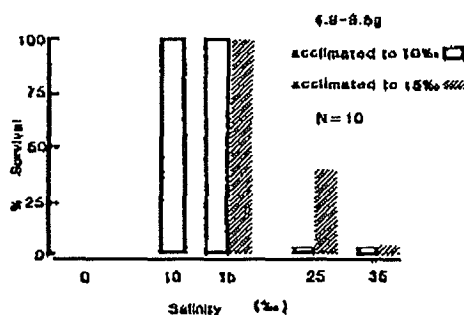


Fig. 2. Percent survival of 4.9-9.5g white sturgeon at various salinities with one week acclimation to either 10 ppt (open bars) or 15 ppt (hatched bars) salinity.

day sea water values of  $\text{Na}^+$ ,  $\text{Cl}^-$ , and osmolality. The  $\text{K}^+$  concentrations of the freshwater acclimated fish compared with the 3 day sea water values were significantly different ( $P < 0.05$ ).

There was a gradual increase in the survival of juvenile *A. transmontanus* after abrupt transfer to 10 ppt, 15 ppt, with increased size (weight) as shown in Figure 1. All fish of all size groups survived transfer to 0 ppt and 5 ppt. Some fish below 1.8g could not withstand abrupt transfer to 10 ppt whereas some fish below 4.9g did not survive transfer to 15 ppt. In addition, no juvenile fish survived direct transfer to salinities of 25 ppt and above. Blood samples obtained from 18-56g fish after nine h in 15 ppt showed a small rise in plasma osmolality from a fresh water mean of  $245 \pm 1$  to  $306 \pm 6$ . In contrast, the plasma osmolality of the sacrificed (but dying) fish at 25 ppt had risen dramatically to  $410 \pm 19$ . Pre-acclimation to 10 ppt for

one week did not increase survival at 25 ppt, or 35 ppt. However, pre-acclimation to 15 ppt for one week did slightly increase survival at 25 ppt, but not 35 ppt (Fig. 2).

## Discussion

The white sturgeon, *Acipenser transmontanus*, is one of twenty-nine extant species of Chondrostei (Nelson 1976). The chondrosteans are the oldest and most primitive of the actinopterygians, and are believed to have evolved at least two hundred million years ago (Nelson 1976), and possibly as long as four hundred million years ago (Løvtrup 1977) from the palaenoscincoids. Doroshov (1985) has divided many of the living sturgeon species into three categories: anadromous, semi-anadromous, and landlocked (Table 3). *A. transmontanus* is a semi-

Table 2. Plasma electrolyte values ( $\bar{X} \pm \text{SD}$ ) of freshwater acclimated *Acipenser transmontanus* in fresh water and during salinity increase to sea water.

	Salinity (Time at that salinity)			
	0 ppt (> several wk)	22 ppt (24 h)	33-35 ppt (<1 h)	33-35 ppt (3 d)
$\text{Na}^+$ (mEq l <sup>-1</sup> )	$131.6 \pm 4.4$	$145.8 \pm 4.4$	$167.0 \pm 18.1$	$200.8 \pm 12.6$
$\text{K}^+$ (mEq l <sup>-1</sup> )	$2.5 \pm 0.7$	$2.4 \pm 0.3$	$2.8 \pm 0.5$	$3.9 \pm 0.5$
$\text{Cl}^-$ (mEq l <sup>-1</sup> )	$110.6 \pm 3.6$	$119.4 \pm 3.0$	$138.9 \pm 14.0$	$169.3 \pm 11.6$
Osmo (mOsm l <sup>-1</sup> )	$246.1 \pm 7.1$	$272.5 \pm 8.9$	$327.0 \pm 27.7$	$385.0 \pm 18.9^*$
Number of fish*	7	4	4	4

anadromous fish, which spends most of its adult life in sea water, but close to shore, and migrates into fresh water to spawn.

Previous studies on sturgeon osmoregulation (Magnin 1962, Urist & Van de Putte 1967, Potts & Rudy 1972) have indicated that sturgeon are hypo/hyperosmotic regulators, as are the euryhaline and diadromous teleosts. Magnin (1962) captured species of sturgeon (*Acipenser oxyrinchus*, *A. sturio*, and *A. fulvescens*), took blood samples, and analyzed the plasma electrolytes. Urist & Van de Putte carried out a similar study on *A. transmontanus* caught in Suisun Marsh and San Francisco Bay. Potts & Rudy (1972) measured  $^{22}\text{Na}^+$  efflux in one *A. transmontanus*, and several *A. medirostris* in sea water, fresh water, and during the transition. Urist & Van de Putte (1967) reported that *A. transmontanus* caught in San Francisco Bay had similar plasma electrolyte and osmolality values to the *A. transmontanus* caught in Suisun Marsh. Our data are in partial agreement with their findings. However, we found a significant difference in the chloride levels between our freshwater and bay water fish, whereas they did not. We also found that both bay water and freshwater acclimated sturgeon had lower osmolalities ( $248.8 \pm 13.5$ ,  $236.1 \pm 7.1$ , respectively) than did their single data points for bay water and Suisun Marsh caught fish (275 mOsm). These discrepancies between data sets may be due

to the fact that our freshwater values came from fish held in fresh water, and not Suisun Marsh, which has salinity variations from 0 ppt to 12 ppt, depending on the season and the yearly rainfall (personal observation). Unfortunately, Urist & Van de Putte (1967) did not report the salinities at which their fish were caught for either the Bay or Marsh. Studies are underway to determine plasma electrolyte values for sea water acclimated sturgeon.

The dramatic rise in plasma electrolytes of the freshwater acclimated fish gradually exposed to sea water is not surprising as this has also been found to be true of diadromous American eels which are not endocrinologically prepared for the transfer from fresh water to sea water (Ball et al. 1971, Forrest et al. 1973a, b). These eels require about ten days after the transfer from fresh water to sea water for the plasma electrolytes to decrease to those of sea water acclimated eels. This is a result of the freshwater fish not having the ion excreting capacity necessary to maintain blood electrolyte values at sea water acclimated values. In eels, the hormone cortisol is responsible for many of the changes which accompany sea water acclimation (Forrest et al. 1973a, b, Hirano 1980) while in salmonids both cortisol and thyroxine are involved in sea water acclimation (Loretz et al. 1982). Sturgeon, like eels and salmonids, are diadromous and undergo mi-

Table 3. Classification of some of the Acipenseridae by spawning habitat. Classification scheme and data from Doroshov (1985).

Classification	Habitat	Spawning location	Species
I. Truly anadromous species	oceans	migrate up rivers to spawn	1. <i>Acipenser oxyrinchus</i> 2. <i>A. sturio</i> 3. <i>A. medirostris</i>
II. Anadromous or semi-anadromous species	associated with estuarine bays or impoundments species 4, 5, 6, can enter sea water in coastal areas; species 6, 7, 8 can form land-locked populations	migrate up rivers to spawn	4. <i>Huso huso</i> 5. <i>Acipenser transmontanus</i> 6. <i>A. guldensiaedii</i> 7. <i>A. nudiiventris</i> 8. <i>A. fulvescens</i>
III. Completely landlocked populations	inhabit large fresh water lakes and reservoirs (species 10, 11); or live in riverine and lake environments (species 12 and 13)	migrate upstream to spawn	9. <i>A. fulvescens</i> 10. <i>A. ruthenus</i> 11. <i>A. baeri</i> 12. <i>Pseudoscaphirhynchus</i> sp. 13. <i>Polyodon spathula</i>

grations from fresh water to sea water at certain times of the year. Because this is a seasonal, and therefore predictable event, it would not be surprising if there is also a hormonal component to sea water acclimation in sturgeon.

Cortisol has been implicated in the ability of both euryhaline (Doneen 1976, Doneen & Berg 1974, Fosskett et al. 1981, Fosskett et al. 1983) and anadromous (Mayer et al. 1967, Forrest et al. 1979a, b) teleosts to adapt to increased salinities. Cortisol appears to act by stimulating morphological changes in the gills of both euryhaline (Fosskett et al. 1981) and anadromous (Doyle & Epstein 1972) fish, as well as in the gut of anadromous fish (Hirano et al. 1975, Hirano & Mayer-Gostan 1976, Hirano 1980). We are currently pursuing studies on the role of cortisol in seawater adaptation in adult and juvenile white sturgeon.

In our study of the development of salinity tolerance in juvenile *A. transmontanus*, we found increased salinity tolerance with size. However, we did not find a critical body size above which salinity tolerance is independent of body size as has been reported for striped mullet (Nordlie et al. 1982). Both starry flounder, *Platichthys stellatus*, and striped mullet, *Mugil cephalus*, showed increased osmoregulatory ability with increased size (Hickman 1959, Nordlie et al. 1982). The salinity tolerance of white sturgeon between 56 g and 15 kg body weight has not been investigated. The minor difference between the freshwater osmolality data in the juvenile sturgeon ( $245 \pm 1$ ) and the adult sturgeon ( $236 \pm 7$ ) in the present study may have resulted from the two different blood sampling techniques used.

Several studies have found body size to be important in the development of salinity tolerance in juvenile salmonids (Parry 1958, 1960, 1961, Conte & Wagner 1965, Wagner et al. 1969, Farmer et al. 1978). Parry (1958, 1960) concluded that body size, and not age, was the crucial factor in the development of salinity tolerance in salmonids. On the other hand Clarke (1982) believes that it is a maturational event rather than body size that is important. He found that the body size at which the five species of salmonids he studied (chum, sockeye, chinook and coho salmon, and steelhead trout)

exhibited 'optimal hypoosmoregulatory capacity' varying from 0.6 g for chum salmon to 35.0 g for steelhead.

If the development of salinity tolerance is dependent on a maturational event, rather than on surface to volume ratio (i.e., body size) then it would be of interest to determine the nature of the maturational events. Since chloride cells are necessary for survival in a hyperosmotic environment (Fosskett et al. 1981, Fosskett & Scheffey 1982), it may be that the development of chloride cells in the gills is one of a series of important maturational events. Presently we are studying the development of chloride cells in juvenile sturgeon and the role of cortisol in the development of this cell type in the gills of juvenile sturgeon.

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